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SHORT REPORT

Heterogeneity and immunophenotypic plasticity of malignant cells in human liposarcomas

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Abstract Liposarcomas are tumors arising in white adipose tissue (WAT) with avidity for local recurrence. Aggressive dedifferentiated liposarcomas (DDLS) may arise from well-differentiated subtypes (WDLS) upon disease progression, however, this key issue is unresolved due in large part to knowledge gaps about liposarcoma cellular composition. Here, we wished to improve insights into liposarcoma cellular hierarchy. Tumor section analysis indicated that the populations, distinguishable based on the expression of CD34 (a marker of adipocyte progenitors) and CD36 (a marker of adipocyte differentiation), occupy distinct intra-tumoral locations in both WDLS and DDLS. Taking advantage of these markers, we separated cells from a panel of fresh human surgical specimens by fluorescence-activated cell sorting (FACS). Based on chromosome analysis and the culture phenotypes of the composing populations, we demonstrate that malignant cells comprise four mesenchymal populations distinguished by the expression of CD34 and CD36, while vascular (CD31+) and hematopoietic (CD45+) components are non-neoplastic. Finally, we show that mouse xenografts are derivable from both CD36-negative and CD36-positive DDLS cells, and that each population recreates the heterogeneity of CD36 expression in vivo. Combined, our results show that malignant cells in WDLS and DDLS can be classified according to distinct stages of adipogenesis and indicate immunophenotypic plasticity of malignant liposarcoma cells.

Introduction

Sarcomas, cancers of connective tissues, are diagnosed in approximately 10,000 U.S. patients annually with a five-year survival rate of only 50% (Anaya et al., 2009). Liposarcomas, including well-differentiated (WDLS), dedifferentiated (DDLS), pleomorphic, and myxoid variants, arise in white adipose tissue (WAT) and are among the deadliest of these tumors (Kooby

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et al., 2004; Lahat et al., 2008). WDLS are more common and characterized by repetitive local recurrence with minimal risk of metastasis. In contrast, DDLS are aggressive malignancies with the capacity for distant spread and lethal outcome even after an initial successful treatment (Kooby et al., 2004; Gilbert et al., 2009). Malignant cells within WDLS and DDLS typically demonstrate amplification of chromosome 12 (12q13–15) the locus of *MDM2*, *CDK4* and *SAS* genes (Weaver et al., 2009). The liposarcoma cell type in which genetic changes first occur is unknown. It is also unclear if WDLS is the predecessor of DDLS or whether these two subtypes, often found within the same tumor, arise independently. To date, the characterization of liposarcoma cells has only been performed following expansion in culture (Peng et al., 2011). Lack of information on the in vivo cellular liposarcoma hierarchy has hampered the understanding of the mechanisms underlying the disease progression.

Investigation of many solid cancers has been facilitated by classifying constituent malignant cells into distinct populations corresponding to the differentiation stages of benign tissue counterparts (Matsui et al., 2004; Tang, 2012). In response to metabolic imbalance, WAT has a capacity to quickly grow in mass, resulting in obesity (Daquinag et al., 2011a; Sun et al., 2011). WAT expansion is as a result of proliferation and differentiation of a progenitor population that is similar to mesenchymal stromal/stem cells (MSC) initially described in the bone marrow (Prockop, 1997; Pittenger et al., 1999; Bianco et al., 2008; Caplan and Correa, 2011). These adipose MSC, termed adipose stromal cells (ASC), serve as progenitors of preadipocytes (Rodeheffer et al., 2008; Tang et al., 2008), ultimately differentiating into white adipocytes, which are large cells accumulating triglycerides in lipid droplets and the main cellular component of WAT (Cinti, 2011; Daquinag et al., 2011a). In addition to ASC, WAT contains endothelial cells and infiltrating leukocytes, which may also contribute to the adipocyte pool in pathological conditions (Daquinag et al., 2011b; Kolonin et al., 2012). Gene expression profiles (Matushansky et al., 2008) and adipogenic potential of liposarcoma cells (Peng et al., 2011) have indicated the mesenchymal origin of liposarcomas, however the possibility of hematopoietic or endothelial cells also undergoing malignant transformation has not been ruled out.

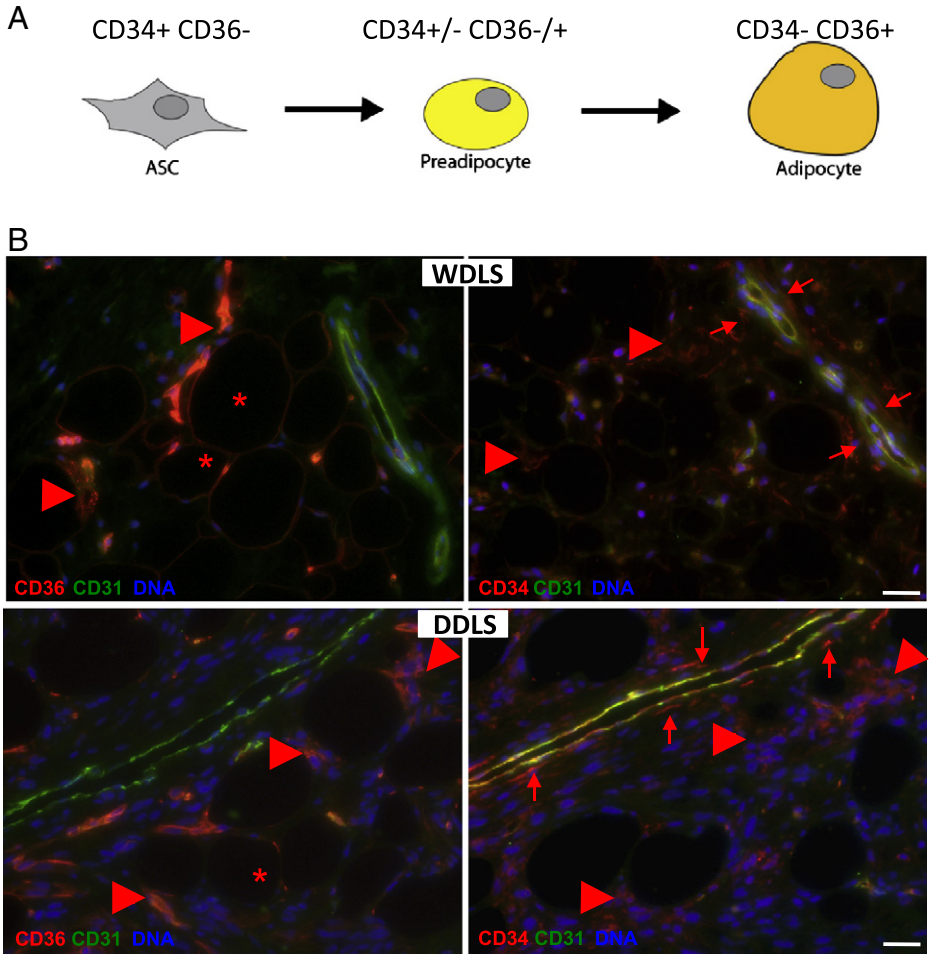

Figure 1 WDLS and DDLS cells at distinct differentiation stages (A) A schematic depicting expression of CD34 and CD36, cell surface markers used for liposarcoma cell classification, during the differentiation of mesenchymal adipocyte progenitors. (B) Immunolocalization of distinct liposarcoma populations in serial paraffin sections of representative WDLS and DDLS samples subjected to immunofluorescence with antibodies against CD36 or CD34 (red). CD36+CD34− cells with adipocyte morphology (*), adjacent stromal CD36+CD34+ cells (arrowheads) and mainly perivascular CD36−CD34+ cells (arrows) are indicated relative to non-malignant vasculature expressing CD31 (green). Nuclei are blue. Scale bar: 50 μm.